4. This work was supported by the department of horticulture, North Carolina State College, Raleigh. The data reported are taken from the Ph.D. thesis of C. H. Hendershott, submitted to the graduate faculty of North Carolina State College in March 1959. We wish to thank Mary Y. Butts, who assisted in this study.
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Inhibitor of O-Methylation of Epinephrine and Norepinephrine in vitro and in vivo

Abstract. Pyrogallol inhibits the O-methylation of epinephrine and norepinephrine by catechol-O-methyl transferase in vitro as well as the metabolism of these catecholamines, and the formation of their O-methylated metabolites, in the intact mouse. Since pyrogallol also prolongs the physiological effects of epinephrine, it is suggested that catechol-O-methyl transferase terminates the actions of the catecholamine hormones.

Previous work in this laboratory has shown that catechol-O-methyl transferase (*1*) is mainly responsible for the metabolism of epinephrine and norepinephrine (*2*). Whether this enzyme inactivates these hormones has not been established. A classical procedure showing a physiological role of an enzyme involves a demonstration that inhibition of the enzyme prolongs the actions of its substrates.

Many years ago Bacq demonstrated that pyrogallol and other catechols markedly increased the duration of response to epinephrine and sympathetic nerve stimulation in vivo (*3*). This potentiation was attributed to the antioxidant properties of the catechols (*3*). Since catechol-O-methyl transferase methylates all types of catechols (*1*), it appeared to us that pyrogallol might prolong the responses of epinephrine by competing for this enzyme.

The action of pyrogallol on catechol-O-methyl transferase was examined in vitro. Incubating epinephrine ($1\times10^{-5}M$) with O-methyl transferase obtained from rat liver, with magnesium chloride, and with the methyl donor S-adenosylmethionine resulted in the formation of metanephrine (3-O-methylepinephrine). In the presence of pyrogallol at a concentration of $1\times10^{-5}M$, the formation of metanephrine was inhibited approximately 50 percent. When the concentration of the substrate was increased 100-fold, the inhibition by pyrogallol was abolished, and thus the competitive nature of the reaction was indicated. Essentially the same results were obtained when norepinephrine was used as a substrate.

The effect of pyrogallol on the metabolism of epinephrine and the formation of metanephrine in vivo was then studied. Mice were given H^3-epinephrine intravenously and were killed 10 minutes later. The whole animal was homogenized in 0.1*N* HCl in a Waring blender, and an aliquot of the homogenate was examined for remaining H^3-epinephrine and for total (free and conjugated) H^3-metanephrine formed (*4*). It was found that about 70 percent of the administered epinephrine was metabolized in this time (Fig. 1). Almost all of the catecholamine that disappeared could be accounted for as metanephrine. Pretreating the mice with pyrogallol dramatically blocked both the metabolism of epinephrine and the formation of metanephrine (Fig. 1), indicating that pyrogallol is an effective inhibitor of catechol-O-methyl transferase in vivo. Pyrogallol also inhibited the disappearance of norepinephrine in the intact mouse. The metabolism of epinephrine was found to be blocked after the intravenous administration of 500 to 10 mg of the catechol-O-methyl transferase inhibitor per kilogram to mice. Epinephrine levels after pyrogallol treatment were elevated for many hours, suggesting that binding of the catecholamine might be an alternate mechanism for the inactivation of this hormone. Although pyrogallol exerts its effects at a relatively low concentration, it is likely that there are more potent inhibitors of catechol-O-methyl transferase. Many flavonoids (rutin, quercitrin) at low concentrations have been shown to potentiate the actions of epinephrine in vivo (*5*). Since flavonoids possess a catechol nucleus, this effect might be due to their ability to inhibit catechol-O-methyl transferase.

For many years monoamine oxidase was considered to be the enzyme chiefly concerned with the metabolism and inactivation of catecholamines. Recent work, however, has shown that the inhibition of monoamine oxidase in vivo did not prolong the physiological effects of exogenous (*6*) and endogenous (*7*) catecholamines. Monoamine oxidase was shown to be mainly involved in the deamination of the O-methylated metabolites of epinephrine and norepinephrine, rather than of the catecholamines themselves (*2*). Treatment with iproniazid, a monoamine oxidase inhibitor, did not affect the rate of metabolism of epinephrine in mice (Fig. 1). These observations provide further evidence for the negligible role of monoamine oxidase in the inactivation of epinephrine.

It has been shown that O-methylation is the principal pathway for the metabolism of epinephrine and norepinephrine (*2*). More recently we have found that within 2 minutes after the administration of epinephrine to cats, more than half of the catecholamine has been converted to metanephrine (*4*). From the observations of Bacq, showing that pyrogallol prolongs the physiological actions of epinephrine (*3*), and from findings that this compound inhibits the O-methylation of the epinephrine and norepinephrine in vitro and in vivo, it can be concluded that catechol-O-methyl transferase is the enzyme primarily concerned with terminating the action of these hormones. The prolongation of responses to sympathetic nerve stimulation after pyrogallol administration (*3*) points to catechol-O-methyl transferase as the enzyme mainly involved in the inactivation of the neurohumor norepinephrine in the sympathetic nervous system.

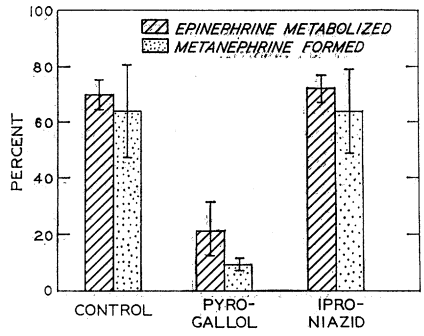

Fig. 1. Inhibition of epinephrine metabolism by pyrogallol. All mice received 3 μg of β-H^3-epinephrine (1.5 μc) in the tail vein and were killed 10 minutes later. Each animal was assayed for H^3-epinephrine and total (free and conjugated) H^3-metanephrine. Pyrogallol was given intravenously (100 mg/kg) 2 minutes before the injection of the epinephrine. Iproniazid (100 mg/kg) was given intraperitoneally 4 hours prior to the administration of the catecholamine. Ten mice were used in each group. The vertical bracketed lines represent the standard deviation of the mean.

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